J. Physiol. (1970), **209**, *pp.* 627–646
With 3 plates and 10 text-figures
Printed in Great Britain

PHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF MOTONEURONES IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH

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(*Received 22 December 1969*)

SUMMARY

1. A number of motor cell bodies have been identified in the segmental ganglia of the ventral nerve cord of the medicinal leech. These motoneurones supply either excitatory or inhibitory innervation to the muscles in the body wall.

2. Several tests were made to establish that each of the identified motoneurones directly innervates muscle fibres. (*a*) By injecting a fluorescent dye into the cell bodies of motoneurones, their axons were traced through one or both contralateral roots. (*b*) Electrical stimulation of a motoneurone by an intracellular electrode caused a single nerve impulse to travel through the roots to the muscles where it set up an excitatory or an inhibitory junctional potential. (*c*) Impulses set up in the roots were conducted antidromically to the cell body. (*d*) If the preparation was bathed in 20 mM-Mg^{2+}, which blocks chemical synapses, conduction from the cell body to the muscles was not interrupted. Thus it is unlikely that an interneurone was interposed in the pathway within the ganglion.

3. Fourteen pairs of excitatory cells and three pairs of inhibitory cells can be identified in each of the twenty-one segmental ganglia. These neurones together supply the five different muscle layers in each segment which execute the movements of the leech. Each neurone innervates a territory of muscle fibres which has a consistent size and location from segment to segment. Several lines of evidence suggest that the identified cells form a major fraction of the total number of excitatory motoneurones in the ganglion.

4. The territories of the motoneurones are arranged in a quilt-like pattern closely resembling that already found for the receptive fields of sensory cells on the skin. Within the longitudinal muscle sheet, individual

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cells supply narrow or wide strips. The firing of each cell thus could produce bending of the segment in various directions or symmetrical shortening of it, depending on which of the motoneurones were active.

5. It is possible to deduce which motoneurones are firing to produce a particular movement of the animal. Thus these experiments provide a basis for studying reflex integration between motoneurones and the identified mechanosensory cells in the ganglion.

INTRODUCTION

The simplicity and segmentation of the medicinal leech make this animal particularly favourable for an investigation of the neuronal connexions underlying reflex behaviour and co-ordination. Apart from specialized head and tail regions, its body is a chain of twenty-one similar units, each containing a group of muscles innervated by a ganglion of the ventral nerve cord. These segmental ganglia are virtually identical, and contain a limited number of neurones (about 350) of which approximately seventy can at present be individually identified. The ganglion has already proved useful for a study of sensory integration; a group of cells comprising the entire rapidly-conducting mechanosensory input from the skin has been found and their receptive fields and central interconnexions have been specified (Nicholls & Baylor, 1968; Baylor & Nicholls, 1969). Any attempt to understand the reflex basis of the animal's movements requires a similar analysis of the motor elements in the ganglion. The work reported here was therefore designed to identify and define the properties of motoneurones which have their cell bodies in a ganglion and supply the muscles of its body segment.

The animal performs a limited number of simple movements. These include 'walking' like an inch-worm, swimming in an undulatory fashion, twisting (e.g. in order to turn over from an upside-down position), and shortening in response to noxious stimuli. These movements are effected by muscle sheets which contract against the hydrostatic pressure of the cylindrical body cavity. Three layers make up the major portion of the body wall and are arranged in circular, longitudinal, and oblique sheets. When the circular muscles contract the animal elongates, whereas contraction of the longitudinal muscles shortens the animal. A fourth group of fibres runs between the dorsal and ventral parts of the body wall and, when contracted, flattens the animal.

In the present paper it will be shown that there are motoneurones which can be consistently identified within each segmental ganglion, and which innervate distinct sectors of these muscle sheets. In the following paper (Nicholls & Purves, 1970), certain functionally significant synaptic

connexions between sensory cells and one of these motoneurones will be described. A preliminary report of this work has appeared elsewhere (Stuart, 1969).

METHODS

Experiments were made on ganglion-muscle preparations from the leech *Hirudo medicinalis*. Earlier papers have described the techniques used in experimentation on leech segmental ganglia (Kuffler & Potter, 1964; Nicholls & Kuffler, 1964; Nicholls & Baylor, 1968). In the present experiments, the preparation consisted of a portion of body wall, removed from the appropriate region of the circumference (e.g. dorsal, lateral or ventral), still innervated by its segmental ganglion. Ganglia were taken from many different positions along the ventral nerve cord, yet there seemed to be no obvious differences between them or the muscles of their segment (the two ganglia near the sexual organs were not used; see Nicholls & Baylor, 1968).

Arrangement for recording from cells, roots, and muscles simultaneously. It was often necessary to record from the roots while bathing the ganglion and the muscles in different solutions. Accordingly, the preparation was mounted in a chamber divided into two compartments by a central oil trough about 1 mm wide. The body wall and ganglion were placed in separate fluid compartments; the root connecting them passed through the oil trough. The walls of the trough were made from thin plastic or a resin (Sylgard, Dow Chemical) into which notches had been cut to accommodate the root. Electrical activity was recorded externally with platinum electrodes from the root in the oil trough.

Criteria for the identification of motor cells are discussed in the Results. Most of the motor cells could be seen when the ganglion was pinned so that it lay flat with its dorsal side facing up. Some of the cells in the anterior packet (see Plate 2) were not readily visible in this position; they were identified and penetrated most easily when they were exposed by making a cut in the connective tissue so that their cell bodies protruded from the ganglion (Kuffler & Potter, 1964).

Either tension or junctional potentials could be recorded from the muscle fibres during stimulation of a motoneurone. When tension was to be recorded, one end of the body wall was pinned to the floor of the chamber. A hook in the other end was tied to a transducer. When junctional potentials were to be recorded, the body wall was pinned flat. The gut side faced upwards for recording from longitudinal and dorsoventral fibres and downwards for recording from circular and oblique fibres (in the latter experiments it was necessary to remove the skin). Micro-electrodes for recording from muscle fibres were filled with 4 M potassium acetate (to prevent reversal of inhibitory potentials; see Baylor & Nicholls, 1969), and had resistances ranging from 50 to 80 MΩ.

Estimation of innervated territories. Boundaries of territories of the motoneurones were defined approximately by watching under the microscope those fibres which contracted in response to stimulation of the cell. The position of contracting fibres was then related to skin markings. For the longitudinal muscles, a light shone upwards through the body wall so that these markings could be seen. Fibres were penetrated with a micro-electrode to confirm that those within the contracting region were indeed innervated while those outside the region were not.

Solutions. Ringer solution had the following composition: 115 mM-NaCl, 4 mM-KCl, 1·8 mM-$CaCl_2$, 10 mM Tris maleate neutralized to pH 7·4 with NaOH, and 12 mM glucose. When necessary, $CaCl_2$ or $MgCl_2$ was substituted for an equivalent concentration of NaCl. Experiments were done at approximately 20° C.

Exploratory experiments to locate excitatory motoneurones were made in Ringer fluid containing 20 mM-Ca^{2+}, since in normal Ringer fluid stimulation of almost any

cell in the ganglion at a sufficiently high frequency will produce a contraction of one or more groups of muscle fibres through polysynaptic pathways. The high calcium concentration raised the thresholds of cells; thus, even though it increased the amount of transmitter released at a synapse, it tended to decrease the probability of synaptic transmission within the ganglion by making it less likely that post-synaptic potentials would cause a cell to fire. The use of high Ca^{2+} Ringer fluid proved to be an effective method for locating possible excitatory motoneurones; in high Ca^{2+}, cells either consistently caused contractions localized to a part of one of the muscle layers, or no movement at all.

Dye injection. A 6% solution of a fluorescent dye (Procion Yellow M4RS) (Stretton & Kravitz, 1968) was electrophoretically injected into cells in order to trace their axons. Cells were injected with pulses of hyperpolarizing current; they usually continued to give action potentials throughout the injection (see Nicholls & Purves, 1970, for detailed methods). The best results were obtained when dye was injected for about 10 min and then allowed to diffuse overnight (about 18 hr) at 4° C. The preparation was then fixed for 1 hr in a mixture of glutaraldehyde and paraformaldehyde (see below). It was dehydrated, cleared in xylene, and mounted in Lustrex. This whole mount of the ganglion was viewed with a microscope equipped with a caesium light source.

Histology. The entire body wall was stretched out and fixed for several minutes at pH 4 in a modified Karnovsky fixative containing 2% paraformaldehyde and 0·12% glutaraldehyde in acetate buffered leech Ringer solution. The wall was then cut into small pieces to allow better penetration of fixative. These were kept in fixative for 5 hr, dehydrated, embedded in Epon, cut into 2–5 μ thick sections, and stained with toluidine blue. Cross-sections of the entire body were also made. A segment was fixed for 24 hr after which it was embedded in Parlodion and cut into 30 μ sections.

RESULTS

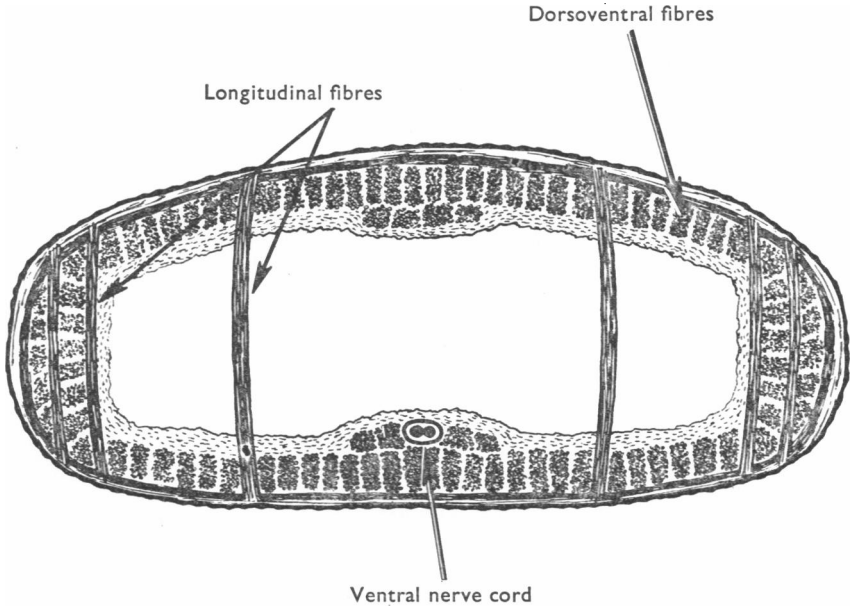
Description of the segmental musculature and ganglion

Body wall musculature. Leech segmental musculature is divided into five groups of fibres which execute the movements of the animal. Pl. 1 *A* and *B* are histological sections showing the three muscle groups, or layers, which constitute the major part of the body wall: (*a*) circular fibres, just beneath the skin; (*b*) oblique fibres (consisting of two layers spiralling around the animal in orthogonally opposite directions); (*c*) the innermost longitudinal fibres. Pl. 1 *A* is a cross-section of the body wall. The circular fibres have been cut lengthwise; the oblique muscles are distinguished by their orientation. The longitudinal muscle layer is the thickest and consists of many rows of fibres arranged in large bundles which are separated from one another by connective tissue.

Pl. 1 *B* is a longitudinal section of the body wall cut across one of the regular circumferential folds, or annuli, in the skin of the animal (thus this section is cut at right angles to that of Pl. 1 *A*). It reveals a fourth layer of muscle fibres (arrows) that were traced in succeeding sections to their insertion into skin on either side of the annulus. The position of these fibres suggests that they are responsible for a movement in which the annuli are

erected into pronounced ridges. The effect of this group of muscle fibres is illustrated in Pl. 1*C*, a photograph of a patch of skin showing five annuli; the middle annulus has been erected into a ridge.

A fifth group of muscle fibres runs dorsoventrally and traverses the body cavity among the viscera. Several of these fibres have been drawn into a diagram of a cross-section of the body wall seen in Text-fig. 1. They form a number of regular rows which, at the lateral part of the animal, lie close together and have actually become incorporated into the body wall.



Text-fig. 1. Diagram of a cross-section of the entire leech body wall. Longitudinal fibres, cut in cross-section, are arranged in bundles separated by connective tissue. Several dorsoventral fibres are shown as they traverse the body cavity. In the lateral part of the body wall, these dorsoventral fibres course among the bundles of longitudinal fibres. Oblique and circular fibres form thin layers under the skin.

The segmental ganglion. A full description of the ganglion has been presented in earlier papers (Coggeshall & Fawcett, 1964; Nicholls & Baylor, 1968). Pl. 2*A* is a photograph of a living ganglion from its dorsal side, where most of the motoneurones lie. All of the cell bodies in the ganglion lie around a centrally located neuropile into which they send a single process and where all the synaptic contacts are made. Identification of individual cells is made easier by septa of fibrous tissue which divide the ganglion into six 'packets' and constitute visible landmarks.

Identification and properties of the motoneurones

In the segmental ganglion, seventeen pairs of motor cells have been found. Each neurone can be consistently identified in any of these ganglia by a variety of criteria, including size and position in relation to other cells and to packet margins. Some cells are unmistakable from these visual clues alone; others are more difficult to recognize and their identification ultimately depends on observing which muscle the cell causes to contract when it is stimulated. The shape of the action potential cannot usually be used as a criterion for the recognition of individual motoneurones as is possible with the sensory cells (Nicholls & Baylor, 1968); the action potential recorded intracellularly is small (less than 10 mV) and rises slowly, presumably because it does not invade the cell body, and is similar in most of the motor cells (see Text-figs. 3 and 4).

In Pl. 2*B* (a duplicate of the photograph in Pl. 2*A*), cell bodies which can be tentatively identified as motoneurones from their position and size have been outlined. (The ultimate identification depends on stimulating the cells and observing the resulting contractions, and was not done in this ganglion.) Most of the motor cells so far identified lie on the dorsal surface, or dorsolateral to the anterior connective; all innervate musculature on the contralateral side of the body wall. They are among the largest (30–60 μ) cells in the ganglion and have resting potentials of about 40 mV. The position of each neurone and the territory of muscle which it innervates will be described in detail in later sections.

To explore the ganglion for cells that might be motoneurones, a preparation was made of a ganglion innervating a large piece of body wall which contained all of the skeletal muscles except for the inner dorsoventral fibres (see Methods and Text-fig. 1). Cells in every region of the ganglion were impaled and electrically stimulated to see if their firing caused peripheral contractions when the entire preparation was bathed in 20 mM-Ca^{2+} Ringer solution (see Methods). Inhibitory cells were located by exploration of the muscle fibres for inhibitory junction potentials correlated with the firing of a neurone. A number of additional experiments were then necessary to prove that each of these excitatory or inhibitory cells was directly connected to the muscle.

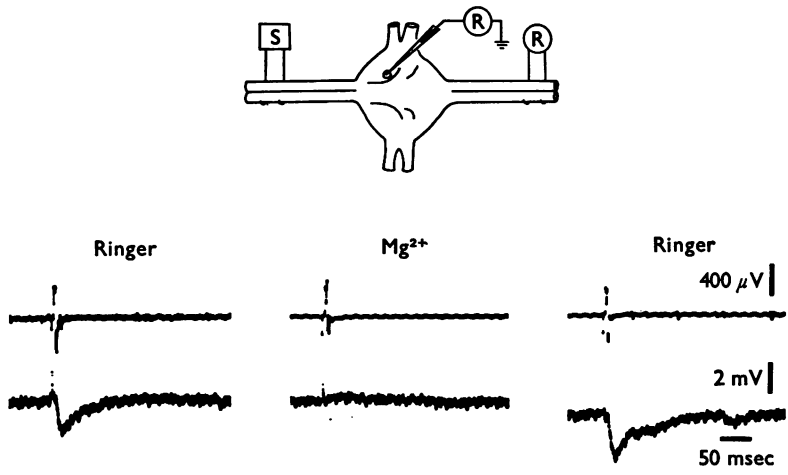
Histological and physiological evidence that the identified cells send axons out of the ganglion. The axon of each motor cell leaves the ganglion through at least one contralateral root. Direct proof of this was provided for several of the neurones by injecting their cell bodies with a fluorescent dye, Procion Yellow, which diffuses extensively throughout cell processes (Stretton & Kravitz, 1968). Pl. 3 shows several photographs of fixed whole mounts in which the processes of injected motoneurones cross the ganglion

and leave through either one (Pl. 3*B*) or both roots (Pl. 3*A*). These processes thus differ from those of the sensory cells which leave by way of ipsilateral roots to supply ipsilateral body wall. A striking feature of the geometry of these motor cells is that each neurone has a principal process which maintains a relatively large diameter as it leaves the cell body and crosses the ganglion, giving off numerous small branches in the neuropile region. This characteristic arrangement has been seen in all of the injected motoneurones and contrasts sharply with the geometry of the sensory cells which have several large processes distributed mainly to the ipsilateral side of the ganglion (Nicholls & Purves, 1970). Pl. 3*C* shows a 10 μ horizontal section through the ganglion at the point of crossing of a pair of injected cells supplying similar muscles on opposite sides of the body. Here the main cell processes come into close apposition.

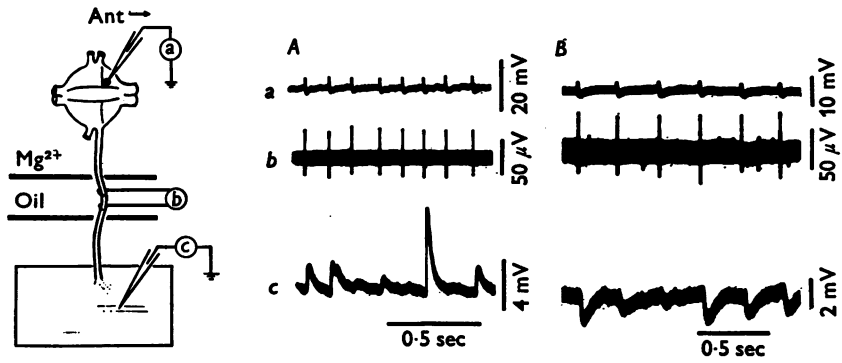
When the cell body of any one of these motoneurones (Pl. 2*B*) was stimulated electrically, an action potential was detected by extracellular recording from the contralateral root. Stimulation of the root was followed by an antidromic action potential recorded in the cell body.

These results show that the cells suspected of being motoneurones send their axons out of the ganglion. It was still necessary to demonstrate that contractions observed in the muscle upon stimulation of one of these cells were not a result of other cells being synaptically driven within the ganglion. To test this, chemical synaptic transmission between neurones within the ganglion was blocked by bathing it (but not the muscles) in Ringer solution containing 20 mM-Mg^{2+}. It is known that high concentrations of Mg^{2+} abolish chemical transmission at many peripheral and central synapses (see e.g. Katz & Miledi, 1963). The following experiments established that this ion has the same effect in leech ganglia. 20 mM-Mg^{2+} abolished excitatory post-synaptic potentials (EPSPs) between sensory cells (Baylor & Nicholls, 1969); it also abolished inhibitory post-synaptic potentials (IPSPs) in a touch sensory cell which consistently followed stimulation of a connective (Text-fig. 2). 20 mM-Mg^{2+} also reversibly eliminated the synaptic activity in the ganglion evoked by delivering a supramaximal shock to all of the axons in a pair of connectives. Finally, all reflex muscle contractions and efferent activity in the root evoked by mechanical stimulation of the skin also disappear in Mg^{2+}. In 20 mM-Mg^{2+}, then, stimulation of a single cell does not activate other cells in the ganglion by way of chemical synapses. This was confirmed by recording electrically from the root a single unitary potential following each impulse in the cell body (Text-fig. 3). The possibility of interaction by way of electrical synapses is mentioned in the Discussion.

Tracing a motor cell to the muscle. Text-fig. 3 illustrates a typical experiment showing that a cell directly innervated a particular group of muscle



Text-fig. 2. Evidence that a high concentration of Mg^{2+} disrupts synaptic transmission in the ganglion. Diagram shows the stimulating and recording arrangement. Upper trace: activity in through-fibres recorded extracellularly from the posterior connectives upon weak stimulation of the anterior connectives. Lower trace: IPSP in a touch cell consistently recorded in response to the same stimulus. The IPSP disappears in 20 mM-Mg^{2+} whereas the action potentials in the through-fibres do not.



Text-fig. 3. Experiments showing innervation of muscle fibres by (*A*) an excitatory and (*B*) an inhibitory motoneurone. A diagram of the experimental arrangement is seen on the left. (*a*) Action potentials in the cell bodies caused by a long depolarizing pulse delivered to the cell through the micro-electrode (current pulse is longer than trace). (*b*) Action potentials in the axon of each cell recorded from the root. (*c*) EJPs (*A*) and IJPs (*B*) recorded from the muscle fibres which each cell innervates. Each action potential in the cell body is followed by an impulse in the root and a junction potential in the muscle fibre, indicating that the cell directly innervates the muscle.

fibres. This cell when stimulated caused contractions of a group of longitudinal fibres. The preparation was arranged as shown in the diagram on the left; chemical transmission in the ganglion was blocked by high Mg^{2+}. A long depolarizing pulse delivered to the cell through the micro-electrode caused it to fire steadily (Text-fig. 3*A*, trace *a*), and the unitary impulses from the cell's axon were monitored with extracellular electrodes placed on the root (trace *b*). A longitudinal muscle fibre was impaled with a second micro-electrode (these fibres usually have resting potentials of about 60 mV). Trace *c* shows that an excitatory junction potential (EJP) occurred with constant latency in the fibre following every impulse in the cell. These junction potentials can be recorded wherever a fibre is impaled within the segment, indicating that the fibres are probably innervated along their length by many terminals from the motoneurone, as in crustacea.

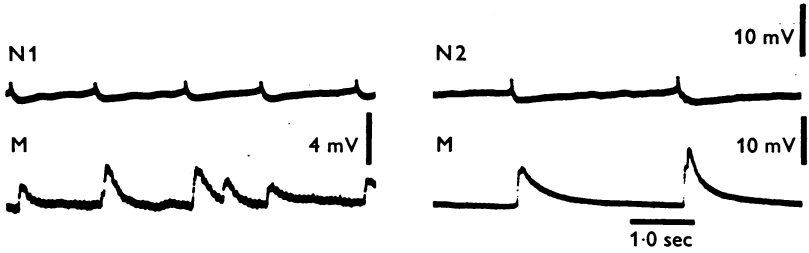
Since no other action potentials can be detected in the root, this experiment provides good evidence that the cell either innervates the muscle fibre directly or is acting on it in a one-to-one manner through a peripheral synapse. Only anatomical studies could eliminate the latter possibility. If such peripheral cells exist, however, they do not participate in local reflexes since removal of the ganglion from a segment abolished all observable reflex responses.

The identity of inhibitory motoneurones was established by similar experiments. In Text-fig. 3*B*, the action potentials from a cell caused inhibitory junction potentials (IJPs) in a dorsoventral fibre (this cell is seen injected in Pl. 3*C*).

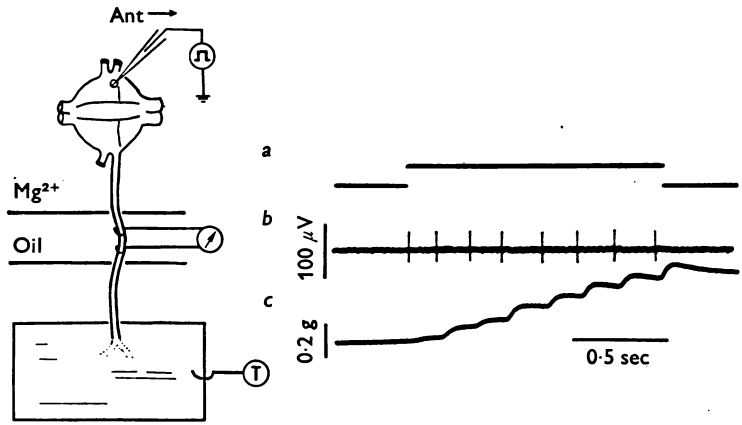
The junction potentials in Text-fig. 3 show large fluctuation in their amplitudes and rise times, and occasionally they fail (see Discussion). In addition, occasional EJPs were unrelated to action potentials in the motoneurone. This raised the question of whether more than one motoneurone could innervate one muscle fibre in regions where territories of the motor cells overlapped. In the experiment of Text-fig. 4, two motoneurones supplying ventral musculature were impaled successively while keeping an electrode in the same muscle fibre during both impalements. One motoneurone (upper left trace), which innervates only a restricted group of muscle fibres in the ventral part of the body wall, caused EJPs in a ventral fibre (lower left trace). The other neurone (the 'large longitudinal motoneurone', upper right race), which innervates all of the longitudinal muscles, caused larger EJPs to occur in the same muscle fibre (lower right trace). All of the ventral fibres impaled during this and similar experiments were innervated by both of these cells.

Recordings were also made of tension produced in the muscle upon stimulation of a motoneurone in the presence of Mg^{2+}. Text-fig. 5 is an

experiment of this type made on the 'large longitudinal motoneurone' where an increment of tension (trace *c*) follows every action potential in the cell (as recorded in the root, trace *b*). This cell was unusual in evoking discrete increments in tension for every cell impulse; most of the motoneurones did not cause tension until the neurone fired at a certain minimum



Text-fig. 4. Innervation of one muscle fibre by two motoneurones. A neurone innervating longitudinal fibres in the ventral part of the body wall (N 1) caused EJPs in a fibre (M). The electrode was then removed from N 1 and placed in the 'large longitudinal motoneurone' which supplies longitudinal fibres over the entire body wall (N 2). The electrode in the muscle fibre (M) remained undisturbed and recorded EJPs correlated with action potentials in N 2. The four additional fibres penetrated in this experiment were all doubly innervated. The preparation was bathed in 20 mM-Ca^{2+} Ringer solution. The 'extra' EJP seen in the muscle recording on the left might have been due to spontaneous activity in yet another identified cell whose territory includes these ventral fibres.



Text-fig. 5. Increments of tension caused in the longitudinal fibres by single action potentials in the 'large longitudinal motoneurone' which supplies the large field extending from mid line to mid line. A diagram of the preparation is shown on the left. On the right, a depolarizing current pulse delivered to the cell body (trace *a*) caused the cell to fire steadily; its action potentials are seen in the root (trace *b*). Each impulse gave rise to an increment of tension (trace *c*). Note the slow relaxation time of these fibres (see Discussion).

frequency. (In leech fibres, as in crustacean muscle, tension depends on the level of depolarization achieved by EJPs in a fibre and thus on their frequency and amplitude.) Nevertheless, tension experiments provided good evidence that a cell was a motoneurone if there was no other activity in the root correlated with the contraction (see Text-fig. 5); such experiments were made primarily in the case of the circular, dorsoventral, and oblique muscles, whose fibres were difficult to dissect and prepare for intracellular recording.

Contraction of the muscle fibres erecting the annuli into ridges was detected by observing the skin through the microscope (see Pl. 1*C*). The identity of the motoneurone to these muscle fibres was confirmed by an additional method. The cell body of the motoneurone supplying these fibres (seen injected in Pl. 3*A* & *C*) was penetrated with a micro-electrode and the root was stimulated extracellularly: when the intensity of this stimulus was increased in small steps, there was a sharply defined threshold at which antidromic action potentials appeared in the cell and the muscle fibres contracted. Further, the range of frequencies which produced different degrees of tension, as determined visually, was the same whether the cell was stimulated in its cell body or via its axon in the root.

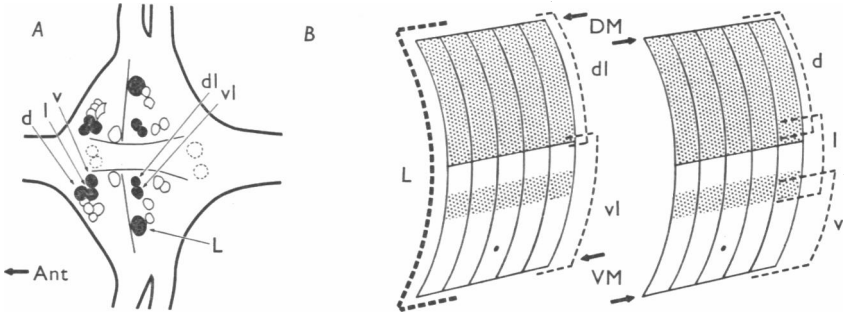
Innervated territories and positions of cells

The skeletal muscles do not operate jointed limbs and have no clearly demarcated boundaries, but instead they are continuous sheets within the body wall. Nevertheless, definite boundaries do exist within a sheet as a result of the innervation pattern of the motoneurones. The area of fibres innervated by a motor cell is conveniently described in relation to the annular markings and longitudinal pigment stripes on the skin, which were the co-ordinate axes used by Nicholls & Baylor (1968) for plotting the boundaries of sensory receptive fields. The extent of a motor territory has been estimated by visual inspection of the contracting fibres.

Several generalizations can be made concerning the arrangement of the motor cell population within the ganglion. Cells innervating the same layer of muscle often lie together in twos or threes. The cells which innervate muscle fibres over the entire extent of the body wall (from mid line to mid line) have branches coursing through both the anterior and posterior roots and the major branch of the posterior root which supplies the dorsum. The remaining cells, which innervate smaller territories, all leave the ganglion through only one of the major trunks. Cells in the anterior part of the ganglion send a process through the anterior root, while those in the posterior part leave through the posterior root.

Excitatory cells innervating the longitudinal muscle. Six of the identified cells provide excitatory innervation to the longitudinal muscles. One of

these cells innervates fibres over the entire contralateral body wall from mid line to mid line (cell L, Text-fig. 6) and is also the largest of the motoneurones. It will be discussed in detail in both this and the succeeding paper (Nicholls & Purves, 1970) and will be referred to as the 'large longitudinal motoneurone'. The other five cells supply restricted areas of the musculature. Three of these five cells always lie together just next to the anterior connective (Text-fig. 6*A*) and are about the same size. Each innervates an approximately equal area of the body wall, dividing it into dorsal,

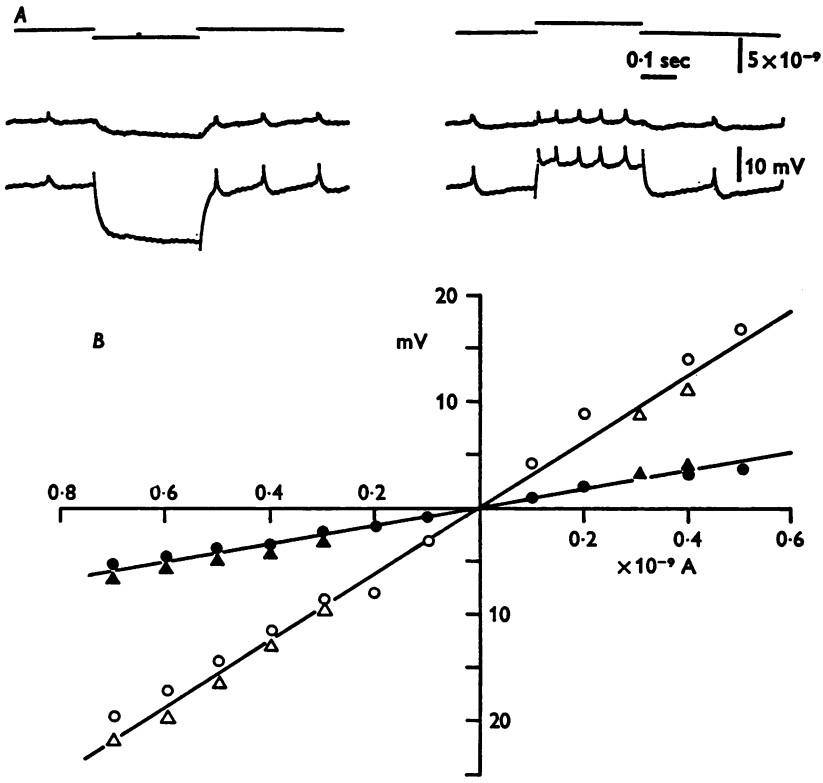


Text-fig. 6. (*A*) Positions in the ganglion of excitatory cells supplying longitudinal musculature. Dorsal aspect of ganglion. (*B*) Approximate circumferential extent of the territories of these cells as related to pigment markings on a piece of skin. DM = dorsal mid line; VM = ventral mid line. Cells are labelled in (*A*) with small letters indicating position of their innervated territory, as seen in (*B*): d = dorsal, l = lateral, v = ventral, dl = dorsolateral, vl = ventrolateral. The cell labelled L innervates fibres from one mid line to the other.

lateral, and ventral regions as shown in Text-fig. 6*B*. The other two are also the same size and lie together within the posterior cluster of cells. They divide the longitudinal musculature into two territories, one dorsal and the other ventral to the lateral stripe in the skin. The extent of all these territories appears to be slightly more than one segment, as determined by intracellular recording.

The 'large longitudinal motoneurone', which innervates a field extending from the dorsal to the ventral mid line, is located at the base of the anterior root. EJPs due to activity in this cell were recorded from all of the contralateral longitudinal fibres that were impaled, indicating that a pair of these cells together innervates all of the longitudinal fibres in the segment. The two cells function as a unit by means of an electrical synapse that synchronizes their activity. Thus, simultaneous recordings from both cells of this pair (Text-fig. 7) showed that an action potential in one cell coincided with either an action potential or a local potential in the other. Passing hyperpolarizing or depolarizing current pulses into either of these

cells caused a measurable voltage change in the other (Text-fig. 7*A*), and this current–voltage relationship was linear up to a displacement of the membrane potential of at least 40 mV (Text-fig. 7*B*). These cells are therefore coupled by a non-rectifying electrical synapse which causes both to

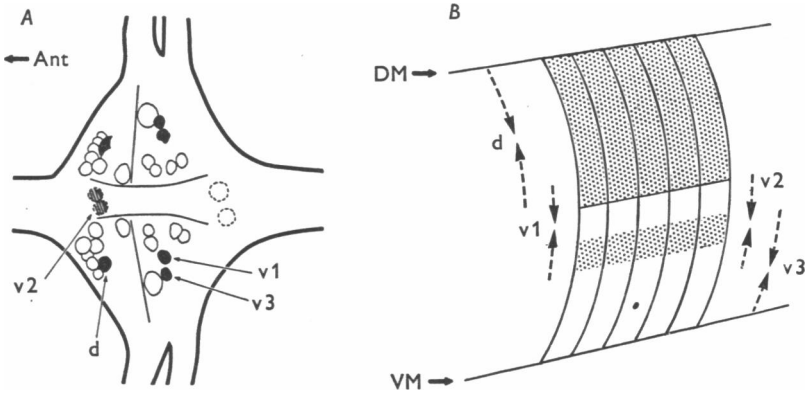


Text-fig. 7. Electrical coupling between the two 'large longitudinal motoneurones' innervating longitudinal muscle from mid line to mid line. Both cells were impaled with micro-electrodes. (*A*) An action potential in one cell is synchronous with either an action potential or a local potential in the other. Depolarizing or hyperpolarizing current pulses delivered to one cell caused a voltage change in the other. This coupling is not abolished by Mg^{2+}. (*B*) Current–voltage relationship from a similar experiment showing that this synapse does not rectify over a range of displacement of the membrane potential of 40 mV. Voltage change recorded in the first cell (△) and in the second cell (▲) in response to current injected into the first cell. Current injected into the second cell gave similar values (○, ●).

fire at approximately the same frequency. This implies that the intact animal would shorten symmetrically in response to the firing of the pair. These cells elicit in the muscle fibres consistently larger EJPs than do the other motoneurones (see e.g. Text-fig. 4), and, as a result, one neuronal action

potential can cause a perceptible increase in tension (see Text-fig. 5). Thus this motoneurone is 'powerful' in that even a low frequency of firing can cause a rapid, strong, contraction of all of the longitudinal muscle.

Cells innervating the circular muscle. There are four cells (see Text-fig. 8) which together cause contractions in circular muscles over the whole extent of the body wall of a segment. Each cell innervates fibres over an extent of nine or ten annuli, including the central annulus of a segment and four annuli on either side of it. Thus, since each segment consists of five



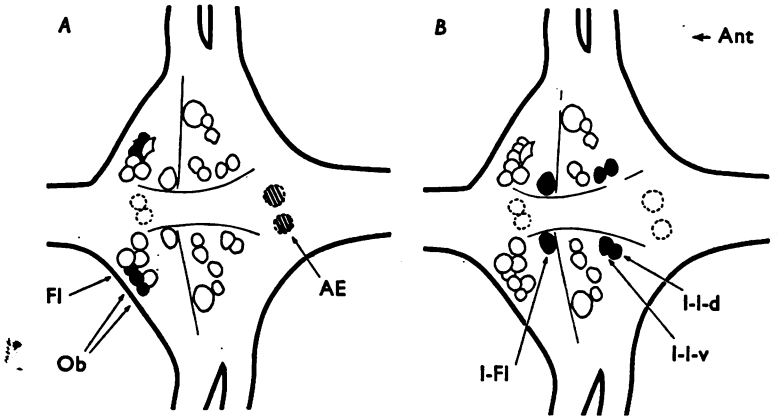
Text-fig. 8. (*A*) Positions in the ganglion of cells that supply circular musculature and (*B*) their innervated regions. Arrows on the skin in (*B*) indicate centre of cell's territory (see text). The ganglion is seen from its dorsal aspect. Abbrevations: d = dorsal, v = ventral, DM = dorsal mid line, VM = ventral mid line. Cells innervating ventral fibres are numbered arbitrarily starting with the most lateral field. Cell v2 lies on the ventral side of the ganglion.

annuli, a cell's territory overlaps that of its neighbours in adjacent segments. This longitudinal extent is the same as that of the 'annulus erector' cell (see below), and is slightly larger than that of a sensory receptive field, which encompasses seven or eight annuli. The territories of these motoneurones were usually determined by watching the overlying skin move instead of trying to expose the fibres themselves. With this technique the centre of a contracting area of circular fibres was obvious (arrows, Text-fig. 8*B*), and although the extent of the area on either side of this centre could not be specified, it appeared to involve an area approximately the length of the arrows in Text-fig. 8*B*.

Cells innervating other muscles. Two small cells lie beside one another in the anterior cluster ('Ob,' Text-fig. 9*A*); each causes contraction in one of the two opposed layers of oblique fibres. The extent of their territories is unknown. Another cell in this cluster ('Fl') innervates the dorsoventral

muscles responsible for flattening the lateral edge of the animal so that it resembles a fin. The longitudinal extent of the field of this cell is also approximately nine annuli.

A single cell at the base of the posterior connective on the ventral side ('AE,' Text-fig. 9*A*) innervates the muscles responsible for erecting the annuli into ridges (Pl. 1 *B* & *C*). Its territory extends from mid line to mid line, and no other cells appear to innervate these muscles. It is called the 'annulus erector' cell and is seen injected in Pl. 3 *A* and *C*. Its territory will be described in detail below.

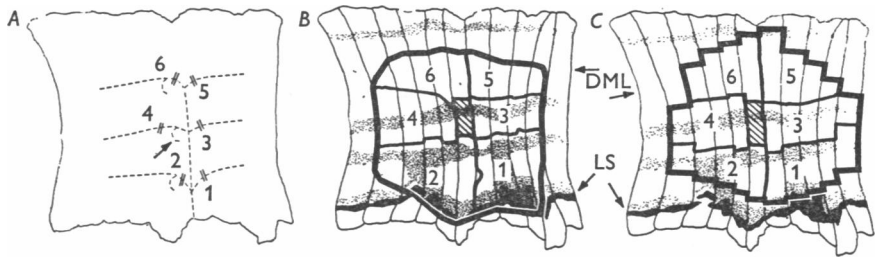


Text-fig. 9. (*A*) Positions of cells innervating oblique (Ob) and dorsoventral 'flattener' (Fl) musculature; also the 'annulus erector' cell (AE), supplying subcutaneous muscle, which lies on the ventral side of the ganglion. (*B*) Inhibitory cells supplying dorsal (I-l-d) and ventral (I-l-v) areas of longitudinal muscle, and dorsoventral 'flattener' fibres (I-Fl). Both ganglia drawn from the dorsal aspect.

Inhibitory cells. So far three cells have been found which provide peripheral inhibition to the muscle fibres (Text-fig. 9*B*). Two of these cells innervate longitudinal fibres; one supplies a ventral and the other a dorsal area. These two cells are of similar size and are located next to one another. The third cell innervates dorsoventral fibres within the body cavity. It has not been ruled out that these cells may in addition innervate fibres in other muscle layers.

The territory of the annulus erector cell. It was natural to question whether the sensory receptive fields in the skin and the motor fields in the muscles had boundaries occurring with similar positions and patterns. Muscles might be innervated in an entirely different manner from skin, which is supplied by *ipsilateral* sensory cells within the ganglion. If any parallels were to exist, it would suggest that similar processes determined the specificity of the sensory and motor peripheral innervation in the animal.

A detailed map was made of the territory of the 'annulus erector' motoneurone, including the way in which the muscles are apportioned among the peripheral branches of the cell. The field of one of these cells covers the entire contralateral half of the segment and is roughly circular; it extends slightly across each mid line, encompassing the central annulus of the segment plus four annuli on either side of it (like the fields of the motoneurones innervating circular muscle).



Text-fig. 10. Innervation of the dorsal body wall by branches of the annulus erector cell and touch cell. (*A*) A diagram of the dorsal branch of the posterior root as it divides to innervate the dorsum. Twigs of this branch were cut in the order as numbered; each cut produced an area of denervation outlined and numbered correspondingly on the maps of dorsal body wall seen in (*B*) for the touch cell and in (*C*) for the annulus erector cell. Cross-hatched area is the portion of body wall which remained innervated by the one intact nerve branch (arrow at left). DML: dorsal mid line. LS: lateral stripe on the skin, dividing dark dorsal skin from lighter ventral skin. Shaded areas indicate pigment stripes.

When peripheral branches of the roots were cut and the area which remained innervated was mapped, it was seen that these branches innervated small areas with surprisingly linear boundary lines, as in the case of the sensory cells (Nicholls & Baylor, 1968). For example, in ten experiments in which the posterior root was cut, the territory innervated by the cell's branch in the anterior root stopped abruptly at the annular margin between the central and the first posterior annulus. This same sharp edge was consistently seen in the case of the sensory cells (Nicholls & Baylor, 1968). Text-fig. 10 shows that the boundaries of the 'annulus erector' motoneurone and the touch cell innervating the same area were similar for regions as small as a fraction of one annulus, indicating that these two cells branched together even in the tiny twigs of peripheral nerve. The photograph of the erected annulus in Pl. 1*C*, taken during a similar experiment, shows the territory of a twig which is confined to a portion of a single annulus. It has not been determined so far whether sensory and motor cells which innervate the same region, but reach the periphery in different nerve roots, also have congruent receptive fields.

DISCUSSION

The present experiments have shown that in the segmental ganglion there are a number of motoneurones supplying the skeletal musculature which have the following stereotyped characteristics: (1) size and position, (2) gross branching pattern of their processes, and (3) area of musculature which they innervate. Thus, each of these motor cells is functionally and anatomically specified, like the sensory neurones in the ganglion (Nicholls & Baylor, 1968), and the motoneurones in the lobster (Otsuka, Kravitz & Potter, 1967).

The identified excitatory cells together provide innervation for all the muscles which the leech uses for its movements. Stimulation of the remaining large cells in the ganglion in Mg^{2+} has so far revealed no additional neurones that cause peripheral contractions. Recordings from the longitudinal muscle fibres rarely showed 'extra' junction potentials that could not be correlated with activity in identified cells. Hence it is unlikely that there are many additional excitatory motoneurones innervating the musculature, although it remains possible that there is additional innervation of the muscles by small unidentified cells. There might however exist inhibitory neurones in addition to the three identified pairs since inhibitory cells are technically more difficult to find.

Several lines of evidence show that all these excitatory and inhibitory cells do directly innervate the muscle fibres. It might at first be thought that sufficient evidence for the motor function of a cell could be established by showing that it causes contractions and sends a process through a root. However, sensory cells in leech ganglia also send processes to the periphery and impulses initiated in these axons also produce consistent and localized muscle contractions through reflex pathways (Nicholls & Purves, 1970).

Membrane properties of motoneurones. The cell bodies of the motoneurones are not actively invaded by the action potential. The impulses in the cell bodies are small and have slow rising and falling phases, so that the point at which active conduction fails must be some distance from the microelectrode. Synaptic potentials recorded in the motor cell bodies often are larger than the action potentials recorded there (see Fig. 2 in Nicholls & Purves, 1970), suggesting that areas of synaptic contact occur closer to the cell body than the site where active conduction of the impulse is initiated. With Procion Yellow, one sees that most of the processes branch off the nerve cell in the large diameter region of the axon as it crosses the neuropile (see cells in Pl. 3). The synaptic contacts on the cell are made here and it is likely that the point of initiation of the action potential is distal to this region. Thus the initial, integrating part of these processes

of the motoneurone within the neuropile might behave in an entirely passive manner.

Electrotonic spread along certain processes in the neuropile must occur with little attenuation since in the case of the electrically coupled 'large longitudinal motoneurones', current injected into one cell body can cause an appreciable voltage change in the other 500 μ away.

Relation of the motor cells to movements of the intact animal. In analysing an animal's reflex behaviour, one would like to be able to specify which cells are firing to produce its movements. The layout of the leech motor system is advantageous for this because, although many of the fields of the motoneurones overlap in part, they are not interspersed among one another. Thus from observing the whole animal's behaviour, one can deduce which of the known motor cells are probably active. In this way, predictions can be made about the central connexions which might exist between motoneurones or between sensory and motor cells (Nicholls & Purves, 1970).

For example, it was predicted that connexions must exist to synchronize the two cells innervating longitudinal muscles from mid line to mid line: It was not clear how discharges in a cell with such a large field could produce a useful movement, such as bending of the animal, so it seemed likely that this cell might control the length of the entire segment. Furthermore, this motoneurone causes considerable tension at a low frequency of firing, which suggested that it could be responsible for rapid shortening, as when the animal withdraws from a noxious stimulus. It was in fact found that a powerful electrical synapse couples this pair of motoneurones so that they fire nearly synchronously, which would produce a symmetrical contraction of the entire animal.

The organization of the fields of the other five cells innervating longitudinal fibres was also predicted as the simplest arrangement which would produce the bending and turning movements of the worm. These five cells subdivide the body wall into longitudinal strips. Contraction of any one of these strips would bend the body in one direction; contraction of two adjacent strips might bend the body in a direction somewhere between them. The over-lap of these strips could account for smoothness in turning and changing directions.

The muscle fibres are diffusely innervated along their length often by at least two excitatory cells and one inhibitory cell. The tension generated in response to stimulation of one motoneurone depends on the level of depolarization reached by the EJPs, and thus on their frequency and amplitude. In these experiments, marked fluctuations occurred in the amplitudes of the junction potentials evoked by one motoneurone. However, these were probably simply a result of recording from a diffusely

innervated fibre at one point; they are understandable if one assumes that the number of quanta of transmitter released by an action potential at individual junctional regions is very low, as in crayfish fibres (Dudel & Kuffler, 1961). Since leech fibres are relatively small (about 20 μ in diameter), they probably have a short length constant so that an intracellular electrode is limited to recording the activity of only the terminals nearest it. If the number of quanta released from these terminals were low, statistical fluctuations would be pronounced and statistical failures of release occurring at junctions near the recording site would enable the smaller and slower rising depolarizations from terminals further away to be seen.

As in other animals with peripheral inhibition of their muscles, it is not really clear how the inhibitory cells fit into the movement pattern. Apart from the obvious assumption that they actually prevent contraction in the muscle, they may serve to modulate movements. For example, peripheral inhibition might be used to speed up the time course of muscle relaxation, as the longitudinal and dorsoventral fibres relax with a time course of tens of seconds after impulses from the excitatory neurone have ceased (see e.g. Text-fig. 5). As indirect support for this idea, the annulus-erector fibres and the circular fibres, to which no inhibitory neurone has yet been found, relax immediately upon cessation of impulses from the excitatory cell.

I wish to thank Dr J. G. Nicholls for essential advice and contributions in all aspects of this work. I am also grateful to Dr D. A. Baylor for many valuable suggestions, and to Dr D. Purves for assistance with the dye injection.

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EXPLANATION OF PLATES

PLATE 1

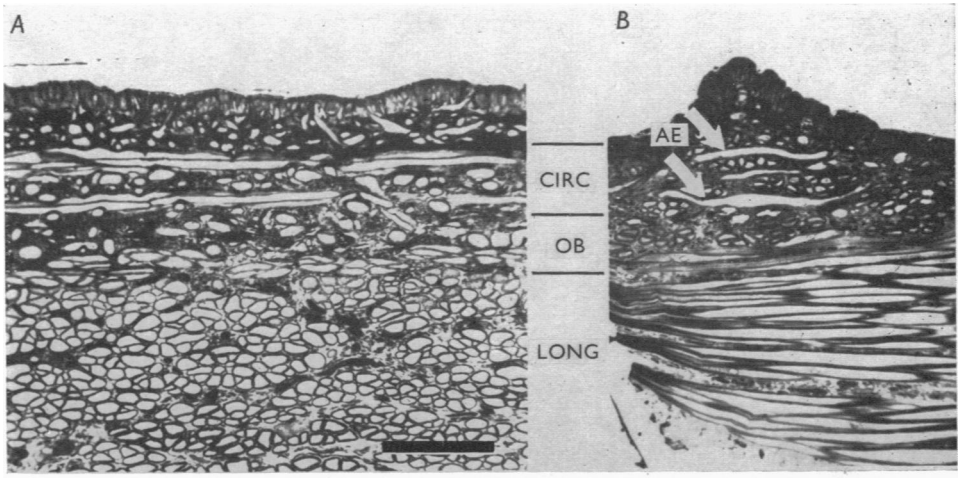
(*A*) and (*B*). Histological sections of the body wall (*A* = cross-section; *B* = longitudinal section; scale = 100 μ) showing circular (CIRC), oblique (OB) and longitudinal (LONG) muscle layers. In (*B*), an annulus is shown slightly raised by the 'annulus erector' fibres (AE), indicated by arrows. (*C*) Photograph of a region of skin showing five annuli; the middle annulus has been erected into a ridge (arrows) by stimulation of the appropriate motoneurone.

PLATE 2

(*A*) is a photograph of a living ganglion seen from its dorsal aspect. Prominent features of the ganglion are labelled and many of the cells can be seen. (*B*) is the same ganglion with cells in the positions of the motoneurones outlined in black. For the most part they lie in two clusters, one lateral to the anterior connective and the other just posterior to a prominent packet margin. Those cells which lie ventrally are drawn in with dotted lines. Scale: 100 μ.

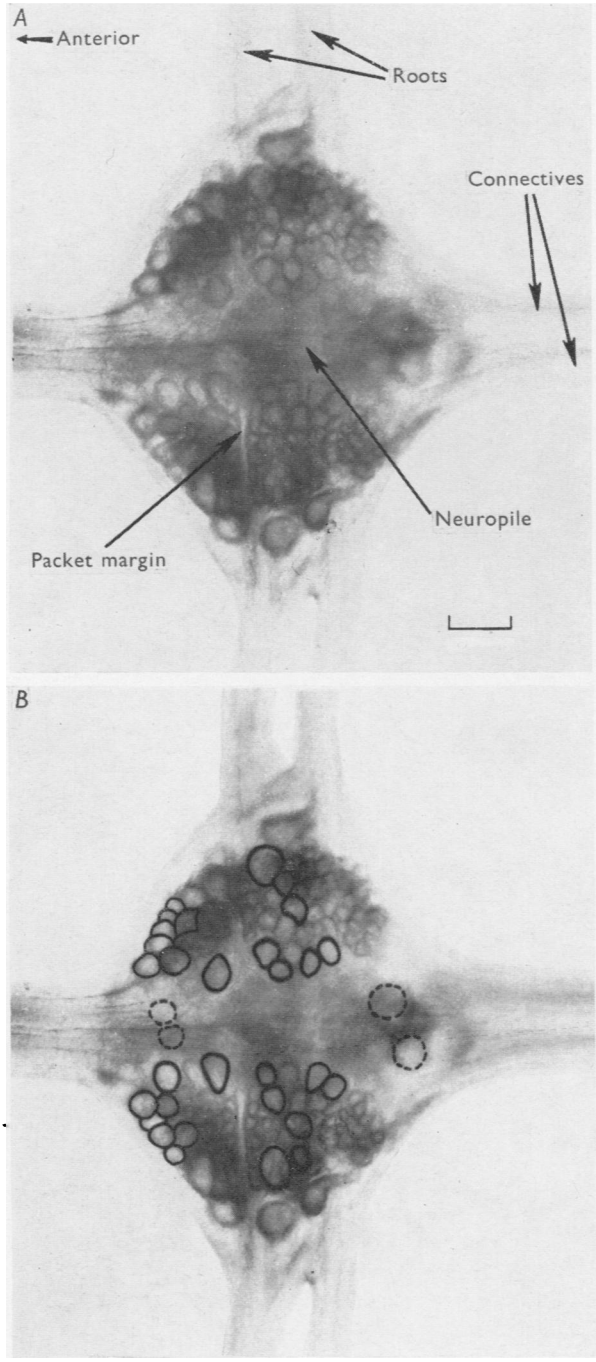
PLATE 3

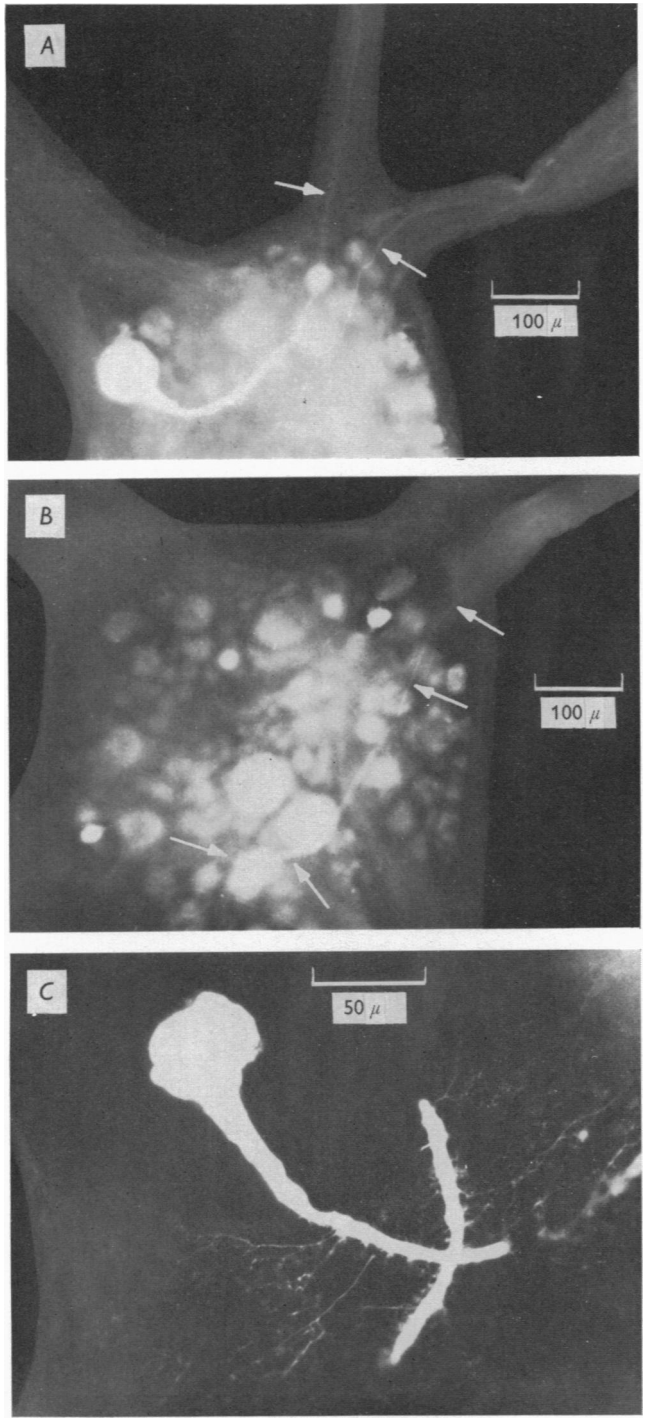
Photomicrographs of motoneurones injected with Procion Yellow M4RS fluorescent dye. (*A*) and (*B*) are whole mounts; (*C*) is a 10 μ section in epon. (*A*) is the 'annulus erector' cell, which sends processes through both contralateral roots (arrows). (*B*) is the inhibitory cell supplying dorsoventral muscles; its axon leaves the ganglion only through the contralateral anterior root (arrows). In the root it passes out of the plane of focus. The two large white cells in this photograph are the Retzius cells, which are naturally fluorescent. (*C*) is the mid line decussation of two injected 'annulus erector' cells. (The soma of one of the cells is not in this section.) All three ganglia are oriented so that posterior is towards the upper left-hand corner.



C

(*Facing p.* 646)


A
Anterior
Roots
Connectives
Neuropile
Packet margin
B


A
100 μ
B
100 μ
C
50 μ